

α -Amylases by strains of *Candida albicans* and *Fusarium sp.*: Expression and characterization

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Abstract: In this investigation, a defined medium was inoculated with *Candida albicans* and *Fusarium sp.* Incubation was at 28°C. The composition of the medium was potassium dihydrogen sulphate, magnesium sulphate, calcium sulphate, hydrated iron sulphate, manganese sulphate, copper sulphate, zinc sulphate, thiamine, biotin, sodium nitrate (nitrogen source) and galactose (carbon source). Proteins which exhibited α -amylase activity were expressed by the isolates within a period of eight days. The enzymes obtained on days of optimum activity were precipitated at 90% saturation and dialysed with buffer. The results showed that, by the seventh day of inoculation of the medium, optimum α -amylase activity was expressed by *Candida albicans*. Production of α -amylase by *Fusarium sp.* was optimum by the sixth day. The α -amylases constituted by the two isolates, partially purified, were stimulated by Na⁺ but inhibited by HgCl₂ and EDTA.

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Key words: α -amylase; *Candida albicans*; *Fusarium sp.*

1. Introduction

Amylases have a three-dimensional structure capable of binding substrate (Lehninger, 1982). The α -amylases could be calcium-containing (Takata *et al.*, 1992; Devlin, 2011). Binding of substrate analogs by α -amylases involve Asp206, Glu230 and Asp297 as participants in catalysis (Kuriki *et al.*, 2005; Voet *et al.*, 2013). However, their substrate-binding sites contain 5 subsites with the catalytic site positioned at subsite 3 (Takata *et al.*, 1992; Voet *et al.*, 2013).

In most countries of the tropics, amylases are imported for wide scale application in the breweries, baking and in detergent production. Source and nature of an enzyme could affect its stability contributing to loss of activity after prolonged storage.

2. Materials and Methods

2.1 Organism and Culture Conditions

The isolate of *Candida albicans* (IBD 112) used in this research study was obtained from the Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria. The organism was isolated from a female patient with Candidiasis. It was subcultured on potato dextrose agar slants and maintained at 4°C. The isolate of *Fusarium sp.* (IFE 008) used was part of the culture collection of Dr. K.O. Awojobi, Department of

Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. It was subcultured on potato dextrose agar slants and maintained at 4°C.

2.2 Growth Medium and Inocula

The isolate of *Candida albicans* was subcultured from potato dextrose agar slants into test tubes of malt yeast extract liquid medium and incubated at 28°C. Ninety-six-hr-old culture at 10⁻² dilution was used as inoculum. One hundred and twenty-hr-old culture of *Fusarium sp.* incubated at 28°C on potato dextrose agar was also inoculum. According to the modified method of Olutiola and Ayres (1973), cultures were grown in a defined medium of the following composition: MgSO₄·7H₂O (0.1 g), CaSO₄ (0.1 g), KH₂PO₄ (0.5 g), MnSO₄ (1 mg), CuSO₄ (1 mg), ZnSO₄ (1 mg), biotin (0.005 mg), thiamine (0.005 mg), FeSO₄·7H₂O (1 mg), galactose (10 g) and Na₂NO₃ (9.9 g) (Sigma) in 1 litre of distilled water. A set of 100 ml of growth medium in conical flasks (250 ml) was inoculated with 1 ml of an aqueous spore suspension containing approximately 6x10⁴ spores per ml of *Fusarium sp.* Another set of 100 ml of growth medium in conical flasks (250 ml) was likewise inoculated with 1 ml of 10⁻¹ dilution of *Candida albicans*. In both sets, experimental flasks contained the inoculated sterilized medium while control flasks contained only the sterilized

uninoculated medium. Spores of *Fusarium sp.* were counted using the Neubauer counting chamber (Olutiola *et al.*, 1991). Experimental and control flasks were incubated without shaking at 28°C (Olutiola and Nwaogwugwu, 1982).

On a daily basis, contents of each flask were filtered using glass fibre filter papers. -Amylase activity in each flask was determined based on the method of Pfueller and Elliott (1969).

The crude enzymes obtained on days of optimum activity were precipitated with ammonium sulphate at 90% saturation and thereafter dialysed with 0.02 M citrate phosphate buffer pH 6.0 containing 5mM sodium azide within 18 hr with several changes of buffer.

2.3 Enzyme Assay

2.3.1 α -Amylase

-Amylase activity was determined using the method of Pfueller and Elliott (1969). The reaction mixtures consisted of 2 ml of 0.1% (w/v) starch in 0.02 M citrate phosphate buffer, pH 6.0 as substrate and 0.5 ml of enzyme. Controls consisted of only 2 ml of the prepared substrate. The contents of both experimental and control tubes were incubated at 35°C for 30 min. The reaction in each tube was terminated with 3 ml of 1 N HCl. Enzyme (0.5 ml) was then added to the control tube. Two millilitre of the mixture from each of the sets of experimentals and controls was separately transferred into new sets of clean test tubes. Three millilitre of 0.1 N HCl was added into the contents of each test tube after which 0.1 ml of iodine solution was added. Optical density readings were taken at 670nm. One unit of enzyme activity was defined as the amount of enzyme which produced 0.01 percent reduction in the intensity of the blue colour of starch-iodine complex under conditions of the assay.

2.4 Characterization of Enzyme

The effect of chemicals on the dialysate of the ammonium sulphate precipitated enzymes obtained on the days of optimum activity were investigated. The ammonium sulphate partially purified dialysed -amylases produced by each isolate on the days of optimum activity were diluted with 0.02 M citrate phosphate buffer pH 6.0 and employed in determining the properties of the enzymes.

2.4.1 Effect of salt

Different concentrations of salt (NaCl) were prepared in starch (0.1% starch in 0.02 M citrate phosphate buffer pH 6.0 containing 5mM sodium azide), such that the final concentrations of salt in the substrate were 2, 5, 10 and 15mM. The preparations

served as substrate for enzyme assay. The reactants were incubated at 35°C for 30 min. -Amylase activity was determined as described.

2.4.2 Effect of specific inhibitors

Different concentrations (2, 4, 6 mM) of ethylene diamine tetra acetic acid (EDTA) and mercuric chloride (HgCl₂) were prepared in starch (0.1% starch in 0.02 M citrate phosphate buffer pH 6.0 containing 5mM sodium azide). These preparations served as substrate for enzyme assay. The reaction mixtures were incubated at 35°C for 30 min. -Amylase activity was determined as described.

3. Results

When a defined synthetic growth medium was inoculated with *Candida albicans* and *Fusarium sp.*, there was evidence of growth of both isolates with mycelia mass within a period of eight days at 28°C. Filtrates from the inoculated medium of both isolates exhibited -Amylase activities. Filtrates from uninoculated medium of either isolate did not possess any detectable -Amylase activity.

-Amylase activity detected in filtrates of growth medium inoculated with suspensions of *Candida albicans*, expressed within eight days is represented (Fig. 1). From the third day of inoculation, -amylase activity increased steadily with optimum activity observed on the seventh day after which there was a decline. Optimum activity was 62.5 units as expressed on the seventh day.

Within eight days, -amylase was detected in filtrates of growth medium inoculated with suspensions of *Fusarium sp.* (Fig. 2). From the second day of inoculation, -amylase activity increased steadily with optimum activity observed on the sixth day after which there was also a steady decline. Optimum activity was expressed as 78.3 units on this sixth day.

3.1 Effect of concentration of salt on α -amylases of isolates

The effect of concentrations of NaCl (0, 2, 5, 10, 15 mM) on the activity of the partially purified -amylase from each isolate was stimulatory. -Amylase activity from both isolates increased as concentration of NaCl increased from 0mM to 15mM. -Amylase activity at 15mM was 68.1 units expressed by *Candida albicans* (Fig. 3) and 89.2 mM expressed by *Fusarium sp.* (Fig 4).

3.2 Effect of concentration of specific inhibitors on α -amylases of isolates

The effect of concentrations of ethylene diamine tetraacetic acid (EDTA) and mercuric chloride (HgCl₂) (0, 2, 4, 6 mM) on the partially purified -amylase activity obtained from the isolates

was inhibitory. -Amylase activity from both isolates decreased as concentrations of both chemicals increased from 0mM to 6mM. Inhibition was to varying degrees.

With mercuric chloride, -amylase activity at 0 mM was 59.2 units for *Candida albicans* but 72.3 mM for *Fusarium sp.* Activity decreased to 21.1 units at 6 mM in our study on *Fusarium sp.* There was

complete inhibition at 6mM in our study on *Candida albicans* (Fig. 5 and Fig. 6). With ethylene diamine tetra acetic acid, -amylase activity at 0 mM was 60.2 units in the study on *Candida albicans* and 72.8 mM in that of *Fusarium sp.* Activity had decreased to 25.6 units at 6 mM in the *Fusarium sp.* study. Activity was 18.1 units at 6 mM with *Candida albicans*. (Fig. 7 and Fig. 8).

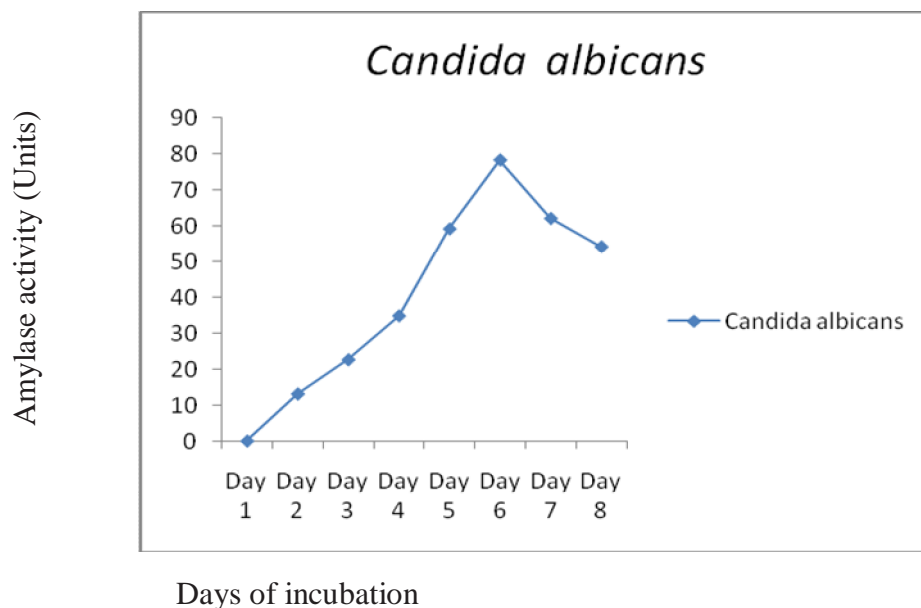


Fig. 1: -Amylase activity produced by *Candida albicans* in growth medium

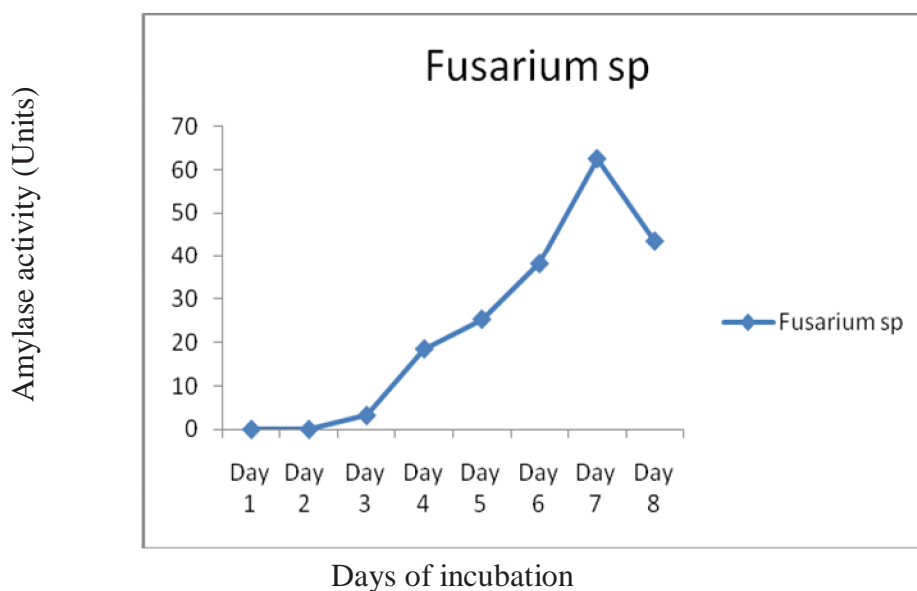
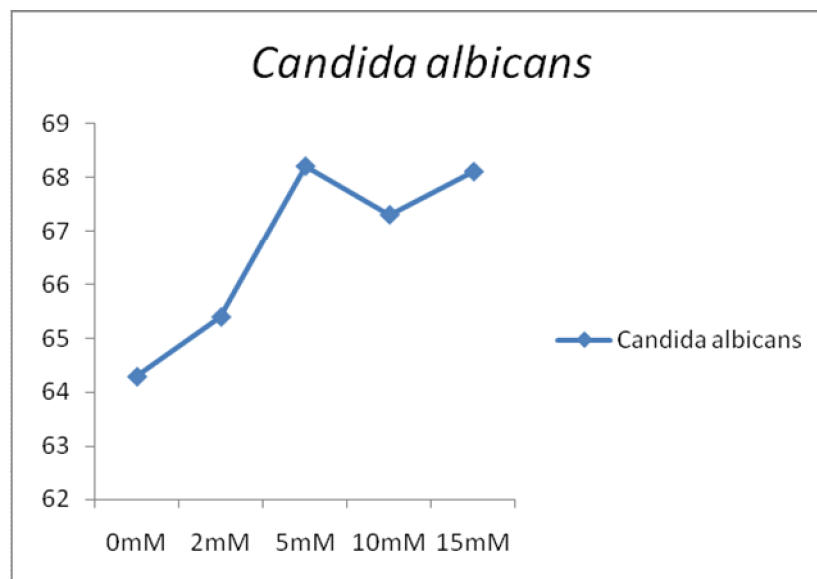
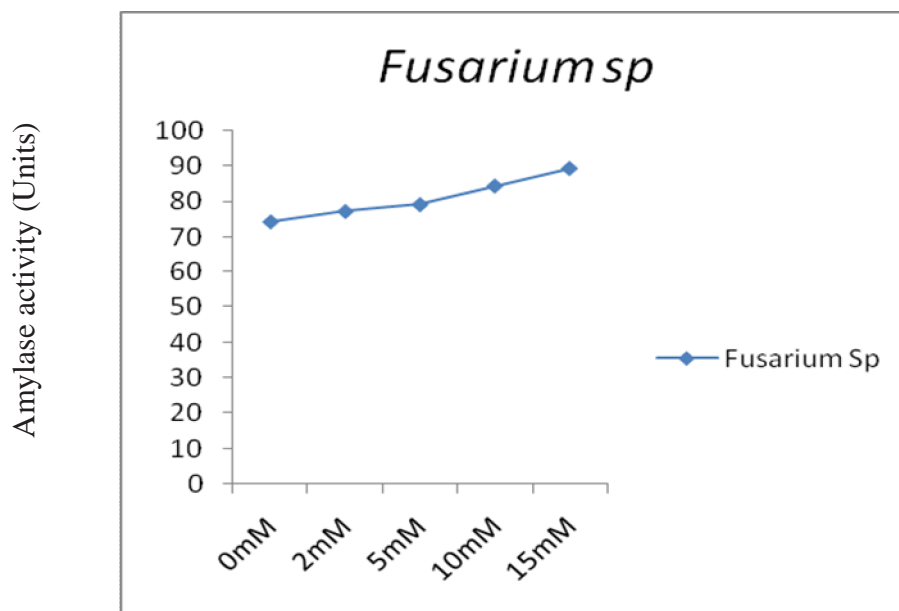


Fig. 2: -Amylase activity produced *Fusarium sp.* by in growth medium



Concentration of NaCl

Fig. 3: Effect of NaCl on α -amylase activity produced by *Candida albicans*



Concentration of NaCl

Fig. 4: Effect of NaCl on α -amylase activity produced by *Fusarium sp.*

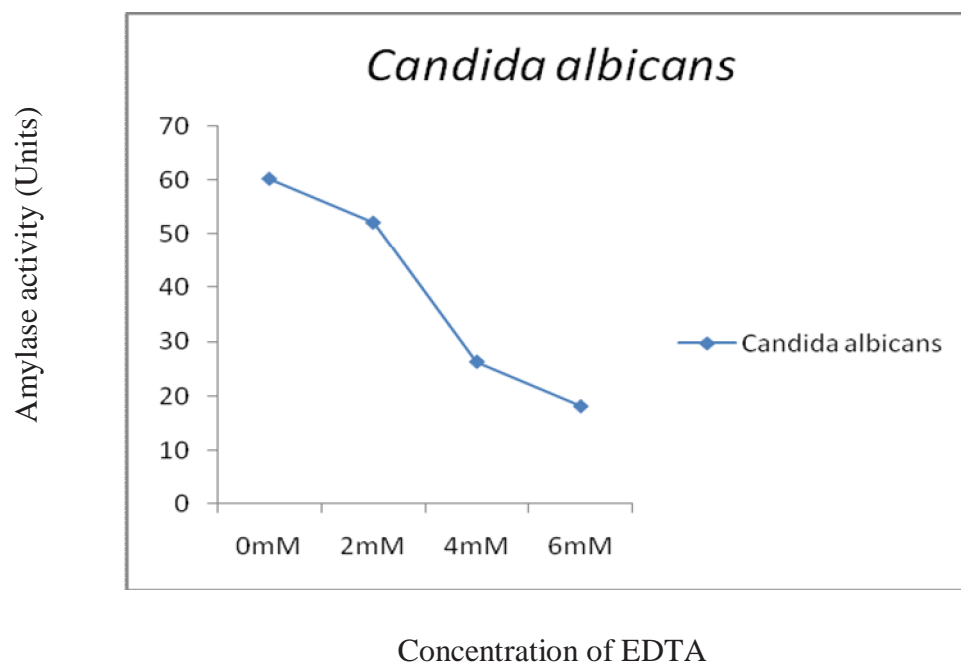


Fig. 5: Effect of EDTA on α -amylase activity produced by *Candida albicans*

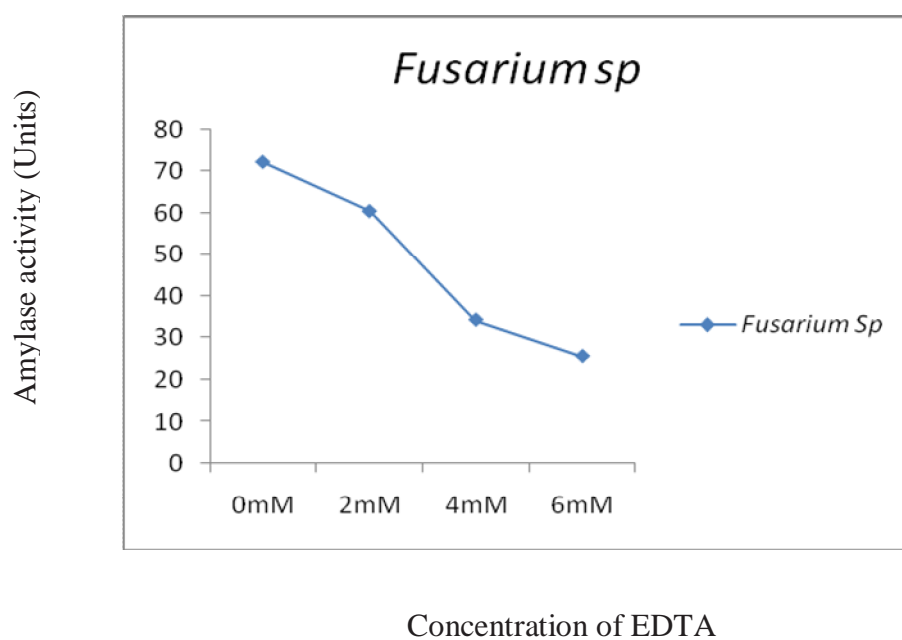


Fig. 6: Effect of EDTA on α -amylase activity produced by *Fusarium sp.*

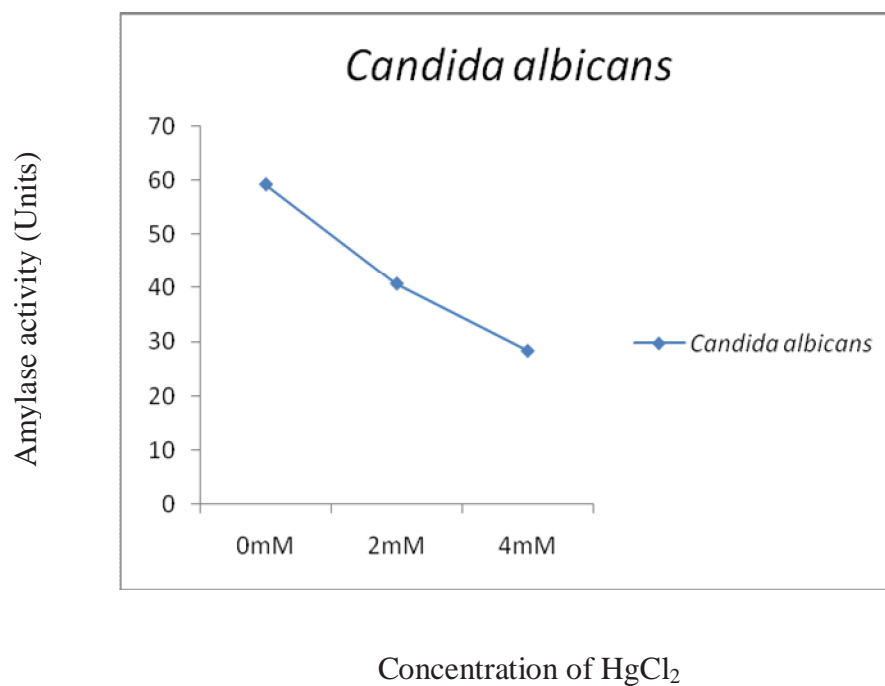


Fig. 7: Effect of HgCl_2 on α -amylase activity produced by *Candida albicans*

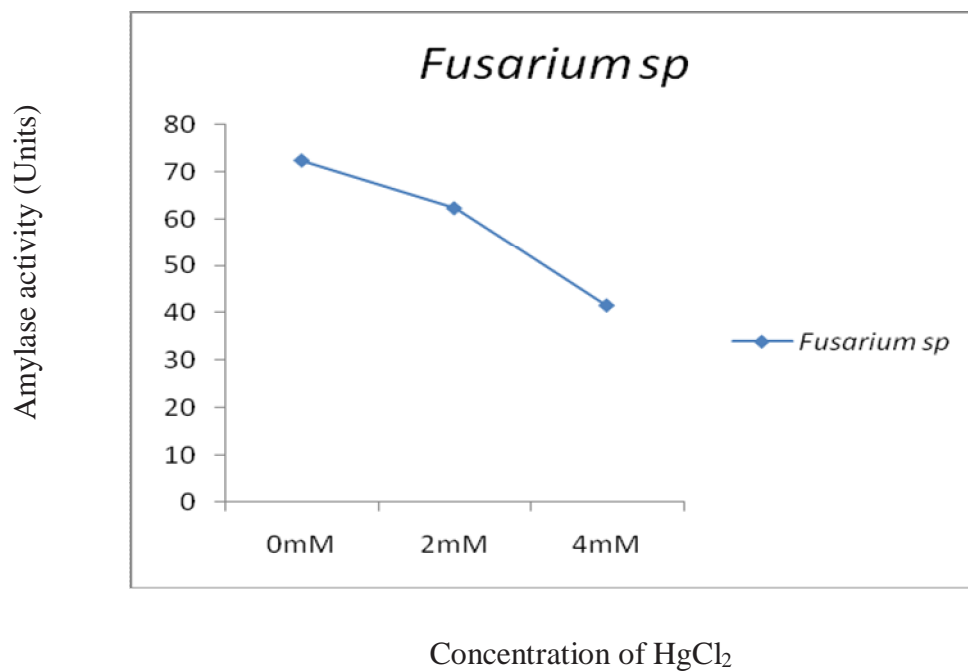


Fig. 8: Effect of HgCl_2 on α -amylase activity produced by *Fusarium sp.*

4. Discussion

As shown in the results, when our defined growth medium was inoculated with *Candida albicans* and *Fusarium sp.*, there was evidence of growth of both isolates by mycelia production within a period of eight days at 28°C. -Amylase production by the two fungi with galactose as carbon source is evidence of constitutive -amylase expression (Voet *et al.*, 2013).

Temperature affects both fungal growth and fungal enzyme. The optimum temperature depends on whether the culture is mesophilic or thermophilic. Among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25-37 °C (Nester *et al.*, 2004; Satyanarayana *et al.*, 2005). Starch degrading -amylase was produced from *Aspergillus niger* at 25°C (Adejuwon *et al.*, 2012). In this study, both fungi were able to grow and produce -amylase at 28°C.

-Amylases from both isolates were stimulated by Na⁺. Adejuwon (2010) reported the production of amylase by *A. niger* isolated from citrus fruit with activity stimulated by sodium ions. *A. flavus* Linn. associated with the green mould rot of yam produced a -amylase in a synthetic growth medium with activity stimulated by both sodium and potassium ions (Adejuwon, 2011a). *Penicillium* species isolated from apple fruit produced amylase stimulated by Na⁺ and K⁺ (Adejuwon, 2011b). Salts of certain metals provide good microbial growth and enzyme production (Nester *et al.*, 2004). Also, ions of certain metals found on the active sites of some enzymes (metalloenzymes) are involved in catalysis. Some are needed for catalysis but not found on such sites (metal-ion-requiring).

Activity of -amylase from both isolates decreased as concentration of heavy metal (HgCl₂) increased. Also EDTA was inhibitory. The effect of inhibition by metal chelators might be the presence of metal ions in the amylases structures required for activity suggesting metallo-amylases nature. Many metal cations, especially heavy metal ions, sulphhydryl group reagents, N-bromosuccinimide, phydroxyl mercuribenzoic acid, iodoacetate, BSA, EDTA and EGTA inhibit enzyme activity (Dixon and Webb, 1971).

4.1 Conclusion

Candida albicans and *Fusarium sp.* can be used in production of -amylases which have potential application in a wide number of industrial processes such as food, fermentation, textile, paper, detergent and pharmaceutical industries. The ability for prolonged survival of some fungi in the tropics could probably contribute to the stability of the enzyme they constitute in such environment.

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